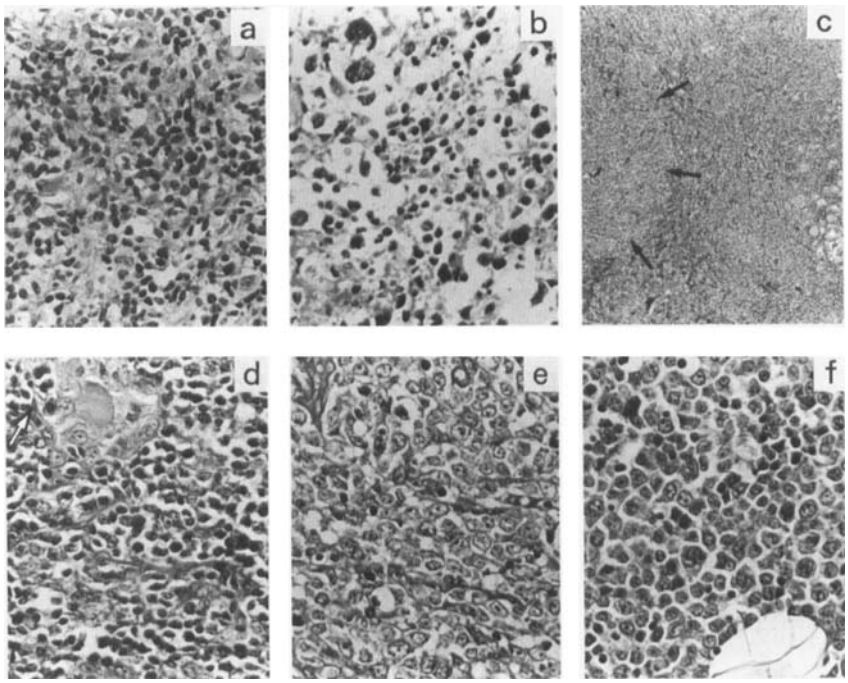

Fig. 1. Histopathological features of biopsy specimens. a, b: Needle biopsy of left thyroid tumor. a: Infiltration of lymphoid cells. b: Giant cells in edematous stroma, with inflammatory changes such as neutrophils and nuclear debris. c: Open biopsy of left thyroid lobe; a focus of lymphoma cells are observed at left (surrounded by arrows), in background of Hashimoto's thyroiditis. d: Higher magnification of lymphoma focus in c, with lymphoepithelial lesion (arrow). e: Tumor in breast at first relapse. f: Subcutaneous nodule at second relapse (H&E stain).

Detection of α-Thalassemia-2 (−3.7 kb) and Its Corresponding Triplication ααα (Anti-3.7 kb) by PCR: An Improved Technical Change

To the Editor: In 1994 we published in this journal [1] a PCR procedure for the detection of the two most common types of α-thalassemia-2, namely, the 3.7-kb and 4.2-kb deletions. This method has since then been used by us and many laboratories around the world with considerable success. Quite a few investigators, however, have experienced some "aspecific" amplification products that have also been observed in our own experiments (Fig. 1, left), and that may interfere with the detection of the 3.7-kb deletion. The reason for the occurrence of these smaller fragments is not clear. In order to avoid this problem we have developed a new set of primers and have used these for more than 1 year for detection of the α-thalassemia-2 (3.7-kb) deletion as well as the ααα (anti-3.7-kb) triplication, without any further complications. The amplification products for the normal, the −3.7-kb allele, and the triplication allele are 1.76 kb long [2], and additional bands have not been observed (Fig. 1, right).

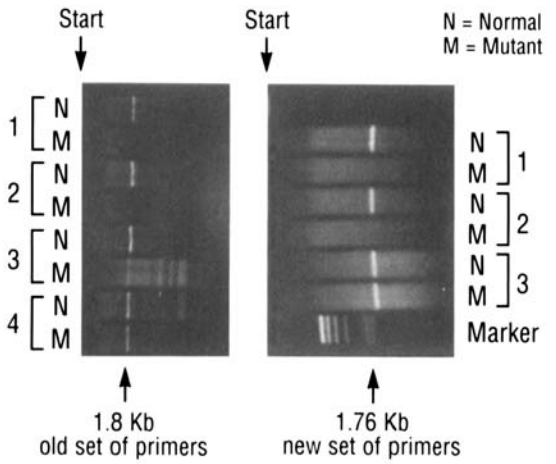

Fig. 1. Detection of 3.7-kb α-thalassemia-2 deletion by PCR. Left: Old procedure; occasionally, smaller-sized amplification products are observed. Right: Similar data with new primer set. Samples 1 and 2, normal (αα/αα); samples 3 and 4; α-thalassemia-2 (−3.7 kb) heterozygote (αα/−α).

Technical details for the detection of the 3.7-kb deletion are as follows: common forward primer (positions +5671–+5695 in the α2 promoter), 5′-CCCTCCCCCTCGCCAAGTCCACCCC-3′; normal reverse primer (positions +7431–+7409 in the 3′UTR of the α2 gene), 5′-GGGAGGCC-CATCGGGCAGGAGGAAC-3′; and mutant reverse primer (positions +11254–+11231 in the 3′UTR of the α1 gene), 5′-GGGGGGAGGCC-CAAGGGGCAAGAA-3′ (positions are listed according to data from GeneBank HUMHBA4). Details for the detection of the ααα (anti-3.7-kb) triplication are as follows: forward primer (positions +9354–+9378 in the α1 promoter), 5′-CCCTCCCCGAGCCAAGCCTCCTCCC-3′; and reverse primer (positions +7431–+7409 in the 3′UTR of the α2 gene), see above.

The 10 × PCR "α" buffer consists of: 1 M Tris-HCl, pH 8.8, 3,350 μl; 1 M $MgCl_2$, 100 μl; 1 M ammonium sulfate, 830 μl; β-mercaptoethanol, 35 μl; bovine serum albumin (4%), 125 μl; and H_2O, 560 μl; final volume, 5,000 μl; the buffer can be kept at 4°C for 2–4 months. The reaction mixture (for two samples) consists of: H_2O, 87.2 μl; 10 × PCR "α" buffer,

11.2 μl; DMSO, 8.6 μl; 6 mM dNTP, 3.0 μl; common forward primer (50 pmole), 2.0 μl; normal reverse primer (50 pmole), 2.0 μl; genomic DNA, 1–4 μl (0.2 μgram). A second reaction mixture for the detection of the mutant allele is prepared the same way, except that the normal reverse primer is replaced by the mutant reverse primer (50 pmole), 2.0 μl. Denature initially for 5–7 min at 99°C; hot-start at 85°C, at which time 2.5 U (=0.5 μl) Taq enzyme (in 5 μl of reaction mixture) are added to each tube. The amplification profile is as follows: denaturation at 95°C (1 min), annealing at 68°C (1 min), and elongation at 72°C (2.5 min) for 25 cycles. It is not advisable to increase the amount of Taq enzyme above 2.5 U. Detection of amplification products is similar to our description in the original paper [1].

As stated, this new set of primers has given us consistently satisfactory data; differentiation between types I and II of the 3.7-kb deletion after *Apa*I digestion of the PCR product can also be obtained with this modified procedure. We have not encountered any such problems with the detection of the 4.2-kb deletion.

N.S. Smetanina
T.H.J. Huisman

Laboratory of Protein Chemistry
Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, Georgia

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Prenatal Diagnosis Based on Simultaneous DNA Analysis for α- and β-Globin Genes

To the Editor: β-thalassemia is the prototype of a single-gene disorder. The majority of β-thalassemia patients show a phenotype which can be directly explained by genotype (severity of mutations). However, a number of clinically significant interactions between α-globin gene abnormalities and β-thalassemia have been described, which can lead to major modifications of phenotype. We report here on the performance of prenatal diagnosis for symptomatic β-thalassemia intermedia, which required analysis-in-parallel of both β-globin and α-globin genes.

Over a decade ago, Wainscoat et al. [1] noted that the phenotype of β-thalassemia patients can be significantly ameliorated by the coexistence of α-thalassemia. On the other hand, symptomatic β-thalassemia has been shown to result from the interaction of heterozygosity for β-thalassemia with excess α-globin genes [2,3]. The carriership of α-globin gene triplication does not affect hematologic parameters. Therefore, individuals who carry this rearrangement will not be identified as at risk for having offspring affected with thalassemia.

A Christian Arab child was referred for molecular diagnosis because of unexplained severe thalassemia intermedia, requiring regular transfusions (once every 6 weeks since the second year of life) for maintenance of normal growth. The father has β-thalassemia trait (Hb 11.5 g/dl, MCV 63.9 fl, MCH 18.9 pg; HbA2, 4.9%), while the mother is hematologically nomal (Hb 13.2 g/dl, MCV 89.8 fl, MCH 29.1 pg; HbA2, 2.9%). The child was found to be heterozygous for β^0-thalassemia, carrying the mutation IVS1 nt1 G-A, which he inherited from his father. Sequence analysis demonstrated that the other β-globin allele was normal. Further investigation showed that his severe phenotype was due to homozygosity for α-globin gene triplication ($\alpha\alpha\alpha^{anti\text{-}3.7}/\alpha\alpha\alpha^{anti\text{-}3.7}$ instead of the normal genotype αα/αα) [2]. Both parents are heterozygous for the α-globin gene triplication. Sequence analysis verified that the mother does not carry any β-globin mutation, and that the father carries the β^0-thalassemia mutation.

During a subsequent pregnancy, prenatal testing was requested by the parents. Fetal DNA was obtained from chorionic villi. Analysis for the IVS1-1 mutation indicated that the fetus was heterozygous (Fig. 1A). Based on this result alone, the fetus would have been diagnosed as thalassemia minor. However genomic α-globin gene analysis of fetal DNA demonstrated homozygosity for α-globin gene triplication ($\alpha\alpha\alpha^{anti\ 3.7}/\alpha\alpha\alpha^{anti\ 3.7}$, Fig. 1B). As the genotype was identical to that of the affected child, the parents elected to terminate the pregnancy.

This case exemplifies a prenatal test which required the analysis of two genes to diagnose what is considered to be a paradigm for a single gene disorder. As the complex factors affecting the phenotype of genetic diseases are clarified, multigene analysis will become more common in the future.

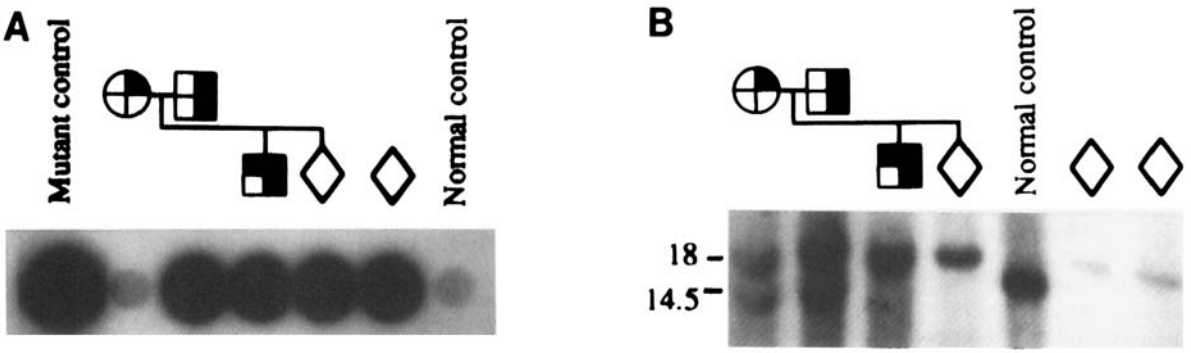

Fig. 1. A: Analysis of the IVS1-1 mutation. DNA isolated from chorionic villi was amplified by PCR using β-globin gene primers [4]. PCR products of the fetus, the propositus, and the parents were dot-blotted together with control samples onto a nylon membrane (Zeta-Probe, Bio-Rad, Hercules, CA), and hybridized to a ^{32}P-labeled IVS1-1 mutant oligonucleotide probe. The two fetal DNA samples shown were amplified separately. $\alpha\alpha\alpha^{anti\text{-}3.7}$ is denoted by stippled symbols, and solid symbols designate IVS1-1 mutation. B: Southern blot analysis of α-globin locus. Fetal DNA obtained from cultured fibroblasts was digested with *Bam*HI, in parallel to DNA of family members and a control. Hybridization was performed with a ^{32}P-labeled α-globin-specific probe [5]. Normal expected fragment size is 14.5 kb (αα). An 18-kb fragment represents a chromosome carrying the triplication ($\alpha\alpha\alpha^{anti\text{-}3.7}$). Three fetal DNA samples were digested and analyzed in parallel. Genotype symbols are as described for A.